510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:

K031016 (bundled)

B. Analyte:

kappa **(κ)** free light chain, lambda **(λ)** free light chain

C. Type of Test:

Quantitative *(nephelometry)*

D. Applicant:

The Binding Site, Ltd.

E. Proprietary and Established Names:

- FREELITE™ Human Lambda Free Kit for use on the Dade Behring Nephelometer™ II *(K010440)*
- FREELITE™ Human Kappa Free Kit for use on the Dade Behring Nephelometer™ II *(K010441)*

F. Regulatory Information:

1. Regulation section:
   21 CFR § 866.5550, Immunoglobulin (light chain specific) immunological test system.

2. Classification:
   Class II

3. Product Code:
   DFH [Kappa (κ)]; DEH [Lambda (λ)]

4. Panel:
   82

G. Intended Use:

1. Intended use(s):
   The FREELITE™ Human kappa **(κ)** free and the FREELITE™ Human Lambda **(λ)** free kits is used to determine the concentration of these light chains in serum and urine on the Dade Behring Nephelometer™ II (BNII). *(The kits are sold separately.)*

2. Indication(s) for use:
   The quantitation of κ and λ free light chains *"…aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldestrom's macroglobulinemia,*

amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythemoatosus."

3. Special condition for use statement(s):
   Not applicable.

4. Special instrument Requirements:
   The test kits are used on the Dade Behring Nephelometer™ II (BNII).

H. Device Description:

Each FREELITE®™ kit contains the specific anti-free light chain antibody, i.e., either anti-κ or anti-λ, and the reagents needed for assaying serum and urine samples. A Dade Behring Nephelometer™ II analyzer, Dade Behring User Defined Reagent Cartridges, Dade Behring Diluents and Buffers, and all associated parts subsequent to loading the calibrator and controls onto the analyzer. *(Each kit is sold separately.)*

I. Substantial Equivalence Information:

1. Predicate device name(s):
   Sebia Hydragel 4IF Kits and Sebia Hydragel 12IF Pentafix Kits

2. Predicate K number(s):
   K960669

3. Comparison with predicate:

Similarities		
Item	**FREELITE® Kit**	**Sebia Hydragel**
Performance	Both test systems detect human immunoglobulin free light chains in serum and urine.	
Differences		
Item	**FREELITE® Kit**	**Sebia Hydragel**
Technological characteristics *	Nephelometry	Immunofixation electrophoresis (IFE)

* Are substantially equivalent *(K010440, K010441).*

J. Standard/Guidance Document Referenced (if applicable):

Not applicable.

K. Test Principle:

The concentration of the soluble antigen is assessed by nephelometry. The test sample is mixed with the appropriate antibody in a solution inside a cuvette. As the antigen-antibody complex forms, a beam of light is passed through the cuvette. The degree of scattering of this light increases with the increase in the concentration of insoluble immune complexes. An excess of antibody is placed in the cuvette so that the amount of immune complex formed is proportional to the antigen concentration. Measurement of the light intensity at an angle away from the incident light is used to monitor the light scattering.

A Calibration curve of measured light scatter vs. antigen concentration is generated using a series of Calibrators of known antigen concentration, and the results read from this curve for samples of unknown antigen concentration.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Repetitive assays of clinical serum at three different levels (low, medium, high) were performed, i.e., 10 runs for within run precision, and a single run on 10 separate assays using the same batch of antisera for between run precision. The data summarized below show consistent results across all three levels with an acceptable percentage Coefficient of Variation (%CV).

Within run Precision:

	κ free light chains			**λ free light chains**		
Level	**1**	**2**	**3**	**1**	**2**	**3**
Mean	15.07	24.88	90.65	18.49	32.18	119.1
%CV	7.22	6.68	4.85	6.53	6.92	5.3

Between run Precision:

	κ free light chains			**λ free light chains**		
Level	**1**	**2**	**3**	**1**	**2**	**3**
Mean	15.54 (N=10)	25.28 (N=8)	103.37 (N=11)	18.06	31.68	111.9
%CV	13.9	10.98	9.69	8.97	5.25	7.19

b. Linearity/assay reportable range:

Linearity was confirmed using serially diluted samples. The regression plot equations where **y** is the measured level of free chain concentration and **x** the theoretical concentration were:

$y = 0.99x - 1.14$ (mg/mL) for **κ** chains

$y = 1.00x - 0.89$ (mg/mL) for **λ** chains

c. Traceability (controls, calibrators, or method):

Not applicable for the purpose of this submission.

d. Detection limit:

The sensitivity when using the 1:100 standard dilution is shown below:

	Serum (1:20)	**Urine (Neat)**
Free κ	1.2 mg/L	0.06 mg/L
Free λ	1.6 mg/L	0.08 mg/L

e. Analytical specificity:

Measurement of purified whole immunoglobulins, i.e., IgG, IgA, and IgM, were used to demonstrate analytical specificity for both test kits. Minimal cross-reactivity was detected, i.e., less than 0.4%.

Interference

Substance	Conc.	Percentage Interference	
		κ (25 mg/L)	**λ (50 mg/L)**
bilirubin	200 mg/dL	2.0	+3.9
haemoglobin	5g/L	+6.8	+6.3
chyle	2.5%	-6.8	-8.1

As shown above, interference of the substances tested was minimal. The values reported in this submission are consistent with the results of similar studies reviewed for clearance of K010440 and K010441.

f. Assay cut-off:

The measuring ranges when using the standard 1:100 sample dilution are 5.9 – 190 mg/L for κ free light chains and 8.1 – 260 mg/L for λ free light chains. *(This is consistent with the previous version of the Package Insert for each kit.)*

2. Comparison studies:

a. Method comparison with predicate device:

The original market clearance for the FREELITE® Human kappa (κ) and Lambda (λ) free kits for use on the Dade Behring Nephelometer II ((K010440 and K01441) was based on a comparison with the Hydragel SEBIA Laboratories® gel electrophoresis specific anti-κ and anti- λ free light chains antibodies immunofixation electrophoresis (IFE) assay (K960669) as the predicate.

In this submission, the applicant provided a full report of the results from the study described below, which was designed to detect free light chains in patients with **primary amyloidosis** and **light chain deposition disease** using the FREELITE® test using nephelometry, and compare these results with those obtained with IFE (i.e., the predicate device used for clearance of K010440 and K010441). [**Note**: Some of the data reported in the 510(k) were published in Abraham et al., (2001) *Clin. Chem*. 47:6, and Katzmann, et al. (2002) *Clin. Chem*. 48:9.]

BRIEF OVERVIEW OF THE STUDY	
Investigational site:	Mayo Clinic, Rochester, MN
Matrices:	Serum *(samples were stored deep-frozen until tested by both methods)*
	Urine *(assayed at time of collection using IF)*
Collection period:	Several years
N:	Amyloidosis *(diagnosis confirmed at collection by immunostaining)* = **95**
	Light Chain Deposition Disease (LCDD) = **18**
Classification:	**Amyloidosis patients**: five groups according to type of Ig Free Light Chain (FLC) and the presence of a monoclonal protein detectable in serum and/or urine by IF *(Each group is described in the results section below.)*
	LCDD patients: four groups according to the similar criteria. *(Each group is described in the results section below.)*
Procedure:	Samples were tested with both the FREELITE ™ Free Kappa and Free Lambda BNII Kits and the predicate device (i.e., IFE). The concentrations of free κ chains, free λ chains, and the free κ/λ ratios were determined.

Results: Primary Amyloidosis

Group	Description @ Serum κ	Serum λ	Urine κ	Urine λ	N	Free κ/λ *	Free κ/λ **	Free κ Concentration	Free λ Concentration
1	+		+		18	13	5	15 were above the 95-percentile range	Mostly within normal range
2		+		+	19	0	19	Mostly within normal range	18 were above the 95-percentile range
3	–		+		20	18	2	18 were above the 95-percentile range	Mostly within normal range
4		–		+	20	4	16 [a]	Mostly within normal range	20 were above the 95-percentile range
5	–		–		18			9 gave abnormal κ/λ ratios and 12 gave abnormal results for free κ and free λ chains, and had abnormal κ/λ ratios.	

@ Presence of monoclonal bands

(*) Number of samples with ratio **above** the range indicated in the package inserts.

(**) Number of samples with ratio **below** the range indicated in the package inserts

[a] Abnormal ratio when compared to the ratio indicated in the package insert

Conclusion: The data show that between 83 and 100% of amyloid patient samples that were positive for either free κ or free λ light chains by serum and/or urine IFE *"…also had abnormally high concentrations of the relevant serum free light chain and/or abnormal free κ/λ ratio*" as determined with the FREELITE ™ test kit, for groups 1, 2, 3, and 4.

Results: LCDD Patients

Group	Description @ Serum κ	Serum λ	Urine κ	Urine λ	N	Free κ/λ *	Free κ/λ **	Free κ Concentration	Free λ Concentration
A	+		+		9 [a]	4 [b]	2 [b]	7 were above the 95-percentile range	Mostly within normal range
B	–		+		4	2	2	4 were above the 95-percentile range	Mostly within normal range
C		+		+	3	0	3	Mostly within normal range	3 were above the 95-percentile range
D	–	–	–	–	3	Normal results			One sample borderline above the normal range referenced

@ Presence of monoclonal bands detected by IFE

(*) Number of samples with ratio **above** the range indicated in the package inserts.

(**) Number of samples with ratio **below** the range indicated in the package inserts

[a] One sample where IFE detected a free κ light chain had a normal κ/λ ratio according to the FREELITE™ assay. The value obtained was borderline and much lower than the value observed for the other eight samples.

[b] Abnormal ratio when compared to the ratio indicated in the package insert

Conclusion: The data show that between 88 and 100% of LCDD patient samples that were positive for either free κ or free λ light chains by serum and/or urine IFE *"…also had abnormally high concentrations of the relevant serum free light chain and/or abnormal free κ/λ ratio*" as determined with the FREELITE ™ test kit, for groups A, B, and C. The IFE method did not detect free chains in the samples in group D. However, either and abnormal (high) concentration of either chain or an abnormal ratio was detected by the FREELITE ™ assay.

b. Matrix comparison:
(Established in original device clearance.)

3. Clinical studies:

a. Clinical sensitivity:
(Established in original device clearance.)

b. Clinical specificity:
(Established in original device clearance.)

c. Other clinical supportive data (when a and b are not applicable):
Not applicable.

4. Clinical cut-off:
The measuring ranges when using the standard 1:100 sample dilution are 5.9 – 190 mg/L for κ free light chains and 8.1 – 260 mg/L for λ free light chains. *(This is consistent with the previous version of the Package Insert for each kit.)*

5. Expected values/Reference range:
The applicant determined value ranges. However, users of the kits should generate their own ranges.

M. Conclusion:

The 510(k) submission complies with all of the required administrative documentation, i.e., a 510(k) statement [as required in 21 CFR 807.93 (a)], a 510(k) Truthful and Accurate statement [as required by 21 CFR 807.87(k)], and the Indications for Use statement [as required by 21 CFR § 807.92 (a) (5)]. *(This information was provided separately for each test kit).*

The purpose of this 510(k) was to modify the last sentence of the indications for use statement for the FREELITE™ Human kappa **(κ)** and the FREELITE™ Human Lambda **(λ)** free kits, used on the Dade Behring Nephelometer II, to add two claims as follows ***(underlined):*** *"Measurement of the various amounts of the different types of light chains aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldestrom's macroglobulinemia,* ***amyloidosis, light chain deposition disease*** *and connective tissue diseases such as systemic lupus erythemoatosus."*

The data submitted in this 510(k) substantiate a high level of agreement between the results obtained with the FREELITE™ test kits (i.e., nephelometry) and the comparison method, i.e., IFE. The data from the new study confirm the level of performance previously demonstrated for both tests (K010440, K010441). Based on the review of the information provided each FREELITE™ kit is as effective as the IFE method in assessing the concentration of either κ or λ free light chains in patients with amyloidosis and LCDD. The FREELITE™ Human Kappa and Lambda Free kits used on the Dade Behring Nephelometer™ II (BNII) are substantially equivalent to the IFE method in this regard. Therefore, the applicant provided

adequate justification for the addition of the two claims indicated above to the Indications for Use statement for each kit.